

Renal Versus Extrarenal Activation of Vitamin D in Relation to Atherosclerosis, Arterial Stiffening, and Hypertension

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Low dietary intake of calcium stimulates the activation of vitamin D₃ precursors to calcitriol in the kidney. This circulating hormone raises blood and urinary calcium by increasing both gastrointestinal absorption of calcium and bone resorption. Renal activation of vitamin D₃ is under tight feedback control. Macrophages also activate vitamin D₃, but, unlike renal tubular cells, they lack feedback suppression of the activating 1 α -hydroxylase. In large-scale epidemiologic studies, blood pressure correlated positively with serum and urinary calcium but inversely with the dietary intake of calcium. Several population-based reports, including the Framingham Study, noticed an association of carotid plaques, arterial calcification, and increased arterial stiffness with lower bone-mineral content. Randomized clinical trials of calcium supplementation did not demonstrate a consistent effect on blood pressure. Macrophages in atherosclerotic lesions can locally activate vitamin D₃ to calcitriol, which might contribute to arterial stiffening and hypertension. Calcitriol acts as a vasoactive

and pro-oxidative substance on vascular smooth muscle cells. In animal models, active vitamin D₃ promotes arterial stiffening and the pathogenesis of systolic hypertension and perpetuates a self-sustaining cycle leading to arterial damage and calcification. On the other hand, active vitamin D₃ inhibits renin activity, thereby decreasing blood pressure in short-term, randomized trials. This article assesses the potential role of active vitamin D₃ in causing cardiovascular complications via its effects on the structure of the arterial wall and the pathogenesis of hypertension. To set the stage and open up new perspectives, our article also summarizes the pathways leading to the renal and extrarenal activation and metabolism of vitamin D₃ and will propose some directions for further research in this complex field. *Am J Hypertens* 2007;20:1007–1015 © 2007 American Journal of Hypertension, Ltd.

Key Words: Vitamin D, macrophages, chronic inflammatory disease, arterial stiffening, atherosclerosis, hypertension.

The roles of calcium and vitamin D₃ in blood-pressure regulation have been widely reviewed,^{1–5} and their effects were assessed in a large number of clinical trials and epidemiologic studies. Epidemiologic surveys in industrialized countries and developing societies revealed an inverse association between blood pressure and the dietary intake of dairy products or calcium.^{6,7} Several large-scale studies showed a positive relationship between blood pressure and either serum total calcium or 24-h calcium excretion. Quantitative overviews of randomized clinical trials of calcium supplementation did not document a consistent effect on blood pressure. Similarly discordant results were reported on the effects of

vitamin D₃,⁸ a key regulator in calcium homeostasis and bone turnover. Accumulating epidemiologic evidence indicates that osteoporosis, obesity, and cardiovascular diseases frequently coexist, suggesting a link between bone, adipose, and vascular tissues.⁹ Higher calcium intake, often specifically from dairy products, in conjunction with normalization of vitamin D₃ status through adequate solar exposure, was conclusively shown to augment bone gain during growth and to slow age-related bone loss and fracture rate.¹⁰ Decreased bone-mineral content is associated with increased arterial stiffness, as assessed by pulse-wave velocity measurements.¹¹ Although numerous population-based studies, including the Framingham sur-

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vey, reported an association of carotid plaques,¹² arterial calcification,¹³ hypertension,¹⁴ and increased arterial stiffness¹¹ with lower bone-mineral content, clinical trials and epidemiologic studies on the role of vitamin D₃ in the pathogenesis of hypertension and arterial disease yielded inconclusive or contradictory results.^{15–17}

The infiltration and accumulation of macrophages in atherosclerotic lesions,¹⁸ and their ability to locally activate circulating precursors of vitamin D₃, might cause deleterious effects on the structure and function of the arterial media by facilitating calcification, leading to arterial stiffening. The administration of pharmacologic doses of vitamin D₃ to rats and rabbits generates reproducible animal models of arterial stiffening and isolated systolic hypertension.¹⁹ Moreover, the progressive shift from the renal to extrarenal activation of vitamin D₃ in chronic inflammatory conditions bypasses the tight renal feedback systems for calcium homeostasis. These long-term processes are confounded by the inhibiting effect of active vitamin D₃ on renin activity, which causes a mild blood-pressure-lowering effect.²⁰

In this article, we assess the potential role of active vitamin D₃ in the causation of cardiovascular complications via its effects on the structure of the arterial wall and the pathogenesis of hypertension. To set the stage for our article, we summarize the pathways leading to the renal and extrarenal activation and metabolism of vitamin D₃. Based on the reviewed evidence, we propose some directions for further research in this complex field.

Renal and Extrarenal Activation and Metabolism of Vitamin D₃

Precursors of vitamin D₃ are generated in the skin from 7-deoxycholesterol by exposure to ultraviolet light in the B-spectrum (UVB). Human food from animal sources and dietary supplements also contain vitamin D₃ precursors (cholecalciferol).^{21,22} Exposure to sunlight will generate approximately 200 IU of cholecalciferol after 10 min of whole-body irradiation. Endogenously synthesized precursors are UVB-unstable and thus inactivated before entering the circulation, preventing an “endogenous overdose” during prolonged solar exposure.²² Cholecalciferol from animal food sources and supplements readily reaches the circulation after intestinal absorption.²¹

The activation of circulating cholecalciferol to the active hormone is biphasic (Figs. 1 and 2). First, hepatic 25-hydroxylase activity converts circulating precursors to calcidiol (25-[OH]-D₃). The principal site of the second hydroxylation, as a result of 1-hydroxylase activity in the proximal tubules of the kidney, is central to the endocrine function of vitamin D₃ as a parathyroid hormone-stimulated modulator of calcium homeostasis.²³ Renal tubular cells express both 24-hydroxylase and 1,α-hydroxylase (Fig. 2). Strict transcriptional feedback mechanisms balance the production of 24,25-(OH)₂-D₃ and calcitriol (1,25-[OH]₂-D₃), the active hormone.²¹ All target cells for

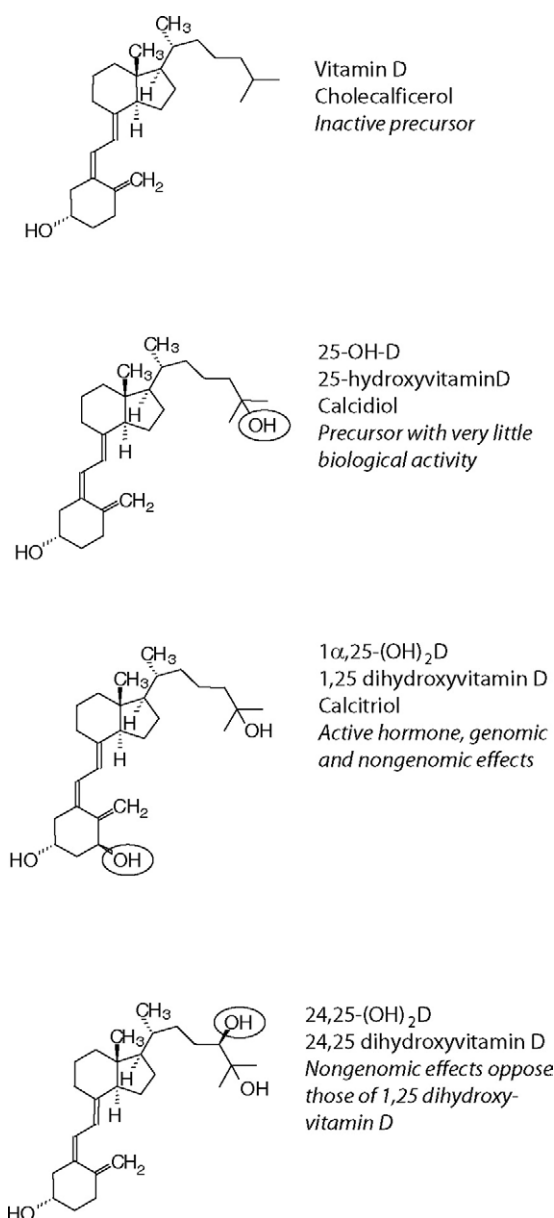


FIG. 1. Structure of vitamin D precursors and metabolites.

vitamin D₃ express 24-hydroxylase, to inactivate calcitriol to calcitroic acid.²¹

Senescence of the skin, defined as changes in pigmentation and thickness, underlies an age-related decrease in endogenous synthesis of vitamin D₃ precursors. Combined with a lifestyle with less solar exposure, it is the main cause of hypovitaminosis D₃ in the elderly, ultimately leading to osteoporosis.^{24,25} On the other hand, the capacity to absorb oral precursors of vitamin D₃ does not decline with age. The administration of vitamin D₃ supplements results in elevated circulating levels of 25-OH-D₃ in both young and older adults.²⁵

The intestinal absorption of orally ingested cholecalciferol is not controlled by feedback. Because of the lack of tight feedback on liver 25-hydroxylase, high oral doses of cholecalciferol induce elevated levels of circulating 25-

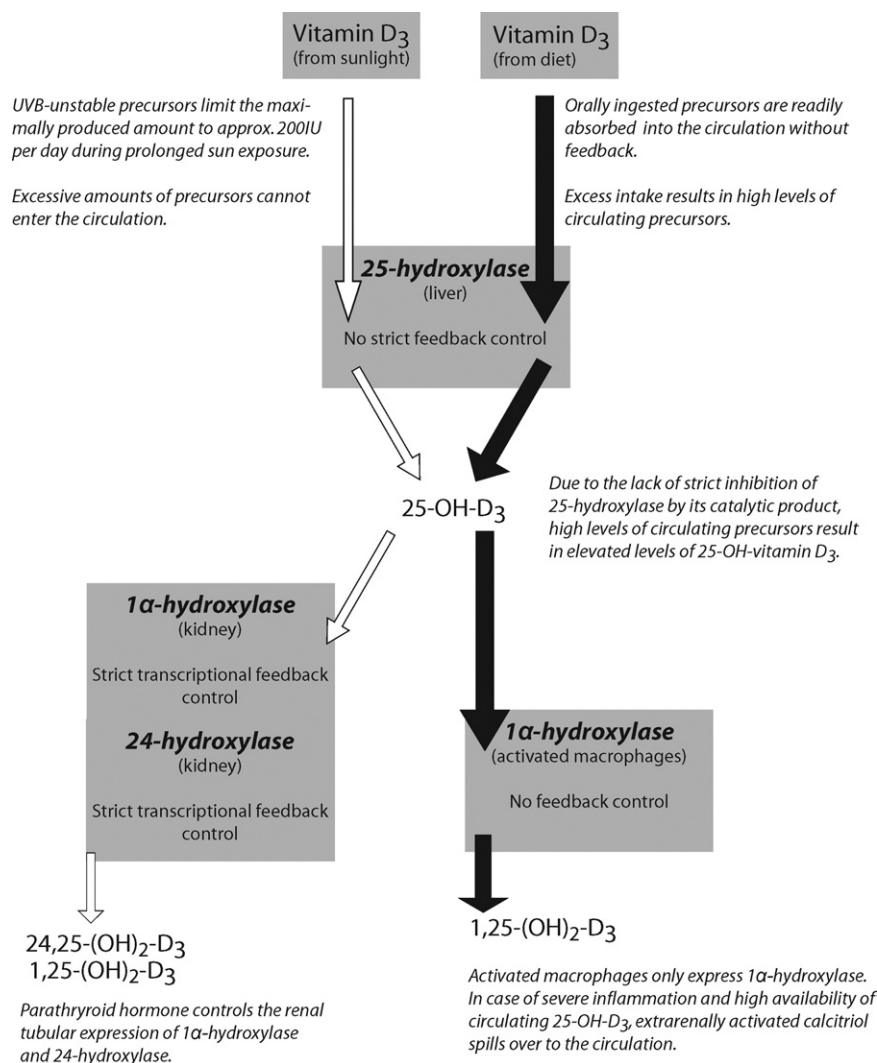


FIG. 2. Pathways in the activation of vitamin D.

hydroxyvitamin-D₃ that can persist for months, thereby maintaining hypercalcemia. In extreme cases, metastatic calcification of the liver and lungs occurs.²⁶ The administration of pharmacological doses of vitamin D₃ to rats and rabbits generates reproducible animal models of isolated systolic hypertension.¹⁹ The underlying mechanism rests upon the upregulation of vitamin D₃ receptors in vascular smooth muscle cells (VSMCs), causing an increased calcium influx, which ultimately leads to disruption of the elastin fibers and calcium deposition in the medial layer of large arteries.²⁷ Bisphosphonates^{28,29} and calcium channel blockers, such as amlodipine, antagonize and can even reverse these arterial calcifications.^{30,31}

Apart from strictly controlled vitamin D₃ activation in the renal proximal tubules, several other cell types can express 1,α-hydroxylase, enabling extrarenal activation of circulating 25-OH-D₃ to the active hormonal form.^{21,32} These include dermal and intestinal epithelial cells, monocytic cell lines, and macrophages.³³ Studies that described the presence and action of vitamin D₃ in primitive organisms such as phytoplanktons prompted speculation as to

the function of vitamin D₃ in these organisms that lack bone tissue.^{34,35}

Extrarenally, in areas of inflammation, macrophages express 1,α-hydroxylase but lack the tight feedback mechanisms present in renal tubular cells (Fig. 2). Only in case of excessive local production does 1,25-(OH)₂-vitamin-D₃ spill over into the general circulation.²⁶ In the 1980s, studies of nonclassical effects of 1,25-(OH)₂-D₃ reported that patients with granulomatous diseases, such as sarcoidosis, had elevated circulating levels of 1,25-(OH)₂-D₃.³⁶ This was due to the extrarenal synthesis of 1,25-(OH)₂-D₃ by activated macrophages.^{37–39} Subsequently, nearly 20 other forms of granulomatous diseases, such as Crohn's disease, were reported to be associated with increased levels of 1,25-(OH)₂-D₃. In these cases, inflammatory activity more closely correlated with the serum concentration of 25-OH-D₃ than with that of 1,25-(OH)₂-D₃. Granulomatous inflammatory processes elevate calcitriol levels and sometimes result in hypercalcemia.^{40,41} Moreover, decreased bone-mineral content is a common finding in these states of chronic inflammation.^{42–44}

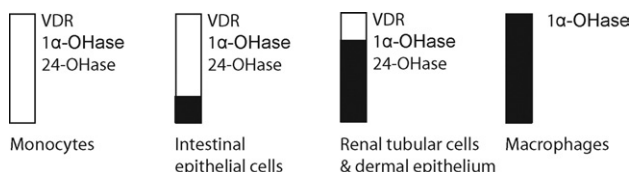


FIG. 3. Expressible vitamin D-metabolizing enzymes and presence of vitamin D receptor (VDR) by cell type. **Solid portions of bars** show level of 1,25-(OH)₂-D production possible per cell type. **Open bar** symbolizes lowest possible level.

Effects of Active Vitamin D₃ on the Arterial Wall

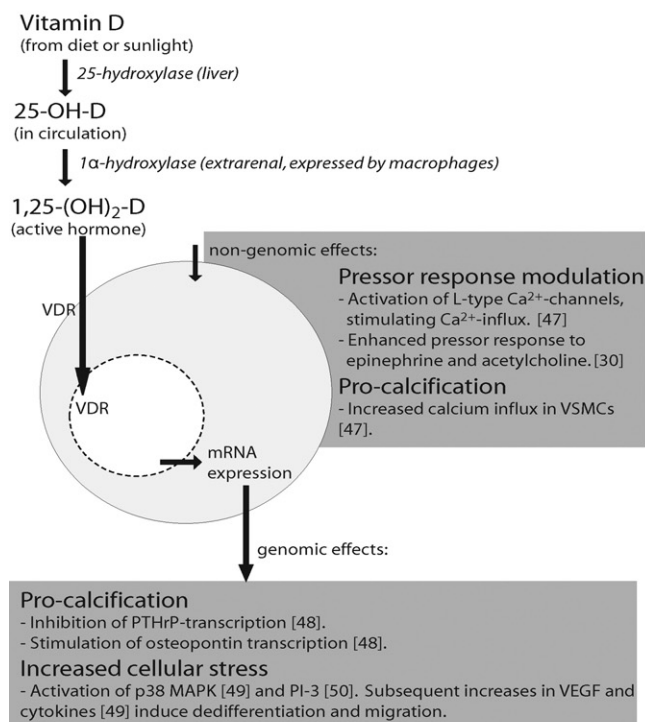
The normal arterial media is a well-organized structure consisting of vascular smooth muscle cells and extracellular matrix proteins. The VSMCs produce elastin and collagens.⁴⁵ They also regulate the active component of vascular tone by varying the diameter of the arterial lumen under the control of circulating hormones, paracrine substances, and neurotransmitters.³⁰ Elastin fibers maintain a continuous blood flow by absorbing energy during systole and recoiling during diastole. They also fulfill a structural role by containing VSMCs in concentric layers.⁴⁵ The integrity of the ring structure of the arterial wall depends on the interaction between fibrillin-1, a protein associated with elastin, and $\alpha_v\beta_3$ integrins expressed on the outer

side of the VSMC membrane.⁴⁵ Nonelastic collagen reinforces the arterial wall, limiting distension at elevated blood pressures. In the wall of atherosclerotic arteries, elastin fibers become fragmented and displaced. Loss of contact between VSMCs and elastin and between $\alpha_v\beta_3$ integrins and fibrillin-1, respectively, leads to dedifferentiation and the migration of VSMCs.^{45,46}

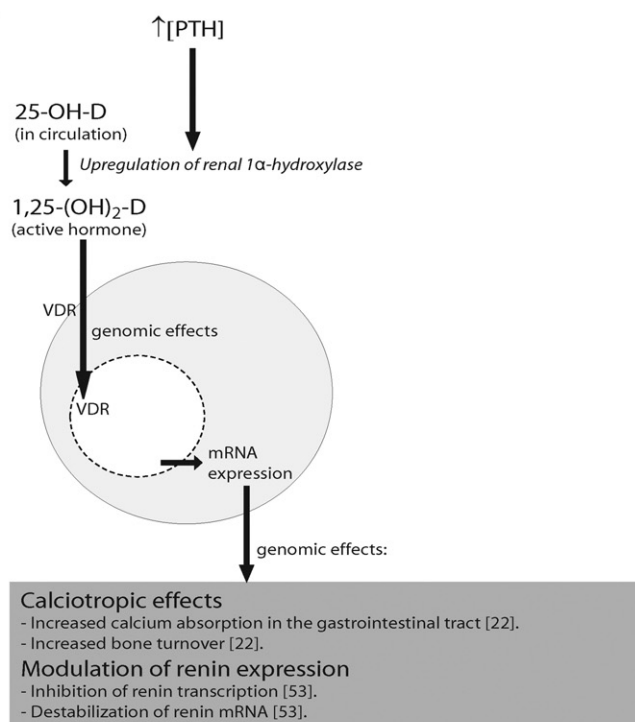
In atherosclerotic lesions, macrophages infiltrate the arterial wall.¹⁸ Their ability to activate circulating precursors to calcitriol can cause deleterious effects on the structure and function of the arterial media. Antigen-presenting cells can act as an abundant source of 1,25-(OH)₂-D₃, depending on the degree of disease activity. Coupled with the lack of 24-hydroxylase feedback regulation, this results in the unregulated production of active vitamin D₃³³ (Figs. 2 and 3).

In vitro studies of VSMCs revealed three pathways via which 1,25-dihydroxyvitamin D₃ might contribute to the pathogenesis of arterial lesions: stimulation of calcium influx,⁴⁷ interference with the basic cellular housekeeping machinery,⁴⁸ and activation of protein kinase-mediated pathways^{49,50} (Fig. 4, left). First, 1,25-dihydroxyvitamin D₃ activates L-type calcium channels.⁴⁷ The resultant inward flow of calcium ions leads to an enhanced contractile response to vasopressors, such as norepinephrine, and promotes calcium deposition on elastin fibers.³⁰ Second,

Local effects on vascular smooth muscle cells



Renally mediated systemic effects



Abbreviations: MAPK: Mitogen-activated protein kinase; PTHrP: Parathyroid hormone—related peptide; VDR: Vitamin D receptor; VEGF: Vascular endothelial growth factor.

FIG. 4. Local and systemic effects of vitamin D.

by inhibiting the transcription of parathyroid hormone-related peptide and stimulating the expression of osteopontin, 1,25-dihydroxyvitamin D₃ promotes calcification.⁴⁸ Third, 1,25-dihydroxyvitamin D₃ dose-dependently activates p38 mitogen-activated protein kinase⁴⁹ and phosphatidylinositol kinase.⁵⁰ Once activated, these signal transducers, in concert with cytokines and growth factors, including angiotensin II, induce cell dedifferentiation, promote cell migration, and increase oxidative stress, thereby leading to the structural disintegration and stiffening of the arterial wall.⁴⁹

Some cross-sectional studies reported an inverse association between coronary artery calcification and the circulating levels of 1,25-dihydroxyvitamin D₃,^{16,17} whereas others failed to demonstrate such an association.¹⁵

Hypertension and Vitamin D₃

The effects of locally activated vitamin D₃ on the arterial wall contradict the acute blood-pressure-lowering effect of oral vitamin D₃ precursor administration as seen in clinical trials. For example, in a randomized clinical trial, supplementation of elderly women with vitamin D₃ precursors for 8 weeks resulted in a significant decrease of systolic blood pressure by 9.3%,²⁰ perhaps because of the inhibitory effect of both 1,25-dihydroxyvitamin-D₃^{51,52} and calcium⁵³ on renin secretion and activity. Cross-sectional surveys noted an inverse association of active vitamin D₃ concentrations and blood pressure.⁵⁴ Recent findings on the modulation of the renin-angiotensin system by calcium and vitamin D₃ set some of the above clinical observations in a more logical perspective (Fig. 4, right).

As reviewed elsewhere,^{5,55,56} several large-scale studies showed a positive relationship between blood pressure and either serum total calcium or 24-h urinary calcium excretion. Acute hypocalcemia produced orthostatic hypotension in human volunteers, whereas an acute elevation of serum calcium induced a parallel rise in peripheral vascular resistance and blood pressure. Recently, Klar et al described the downregulation of renin expression in juxtaglomerular cells by calcium. An increase of cytosolic calcium inhibits renin gene transcription and destabilizes renin mRNA.⁵³

Resnick and Laragh⁵⁷ and Resnick et al⁵⁸ described differences in the serum concentrations of calcium, parathyroid hormone, and active vitamin D₃ levels between low-renin and high-renin hypertensive patients. Short-term administration of the hormonally active form (calcitriol) resulted in a pressor response in low-renin hypertension and a decrease in blood pressure in subjects with high renin levels. However, active vitamin D₃ given over a period of 6 months yielded a decline in blood pressure in low-renin hypertensive patients.^{57–59} Calcitriol administration in high-renin hypertensives in a clinical trial by Burgess et al produced similar findings, leading to the hypothesis that 1,25-dihydroxyvitamin D₃ might be a me-

diator of the response of high-renin hypertensive patients to high sodium intake.⁶⁰

Twenty years later, Li et al⁵¹ described increased renin transcription in vitamin D receptor-null mice. Inhibition of 1,25-dihydroxyvitamin D₃ synthesis in wild-type mice increased the expression of renin, whereas the injection of active vitamin D₃ down-regulated renin transcription. Some vitamin D₃ analogs inhibit renin gene transcription without a pronounced calcemic effect and were proposed as novel antihypertensive compounds.⁵²

Perspectives for Further Research

In cases of local activation of vitamin D₃ by macrophages, spillover to the circulation only occurs during massive overproduction.³³ Interpersonal variations in skin pigmentation, geographic latitude, habitual and seasonal sun exposure, dietary habits, and the use of anti-inflammatory and lipid-lowering medications all have an influence on vitamin D₃ levels.⁶¹ These factors would most likely confound the long-term associations between cardiovascular disease and serum levels of vitamin D₃ and probably explain the null findings of some recent longitudinal studies.^{7,8}

Animal experiments, over and beyond those already published,^{19,27,28} are unlikely to generate more relevant results. Indeed, in short-lived rodents, pharmacologic doses of vitamin D₃ are required to mimic age-related arterial structural damage and calcification, which in humans occur over many years, and usually decades.^{19,30} It is also impossible to measure in vivo the local concentration of 1,α,25-dihydroxyvitamin D₃ in the arterial wall. In arthritic patients, macrophages in synovial fluid locally produce biologically relevant concentrations of active vitamin D₃. In contrast to atherosclerotic lesions, 1,α-hydroxylase activity can easily be assessed in aspirated synovial fluid.⁶²

Preparations with vitamin D₃ precursors are widely available as over-the-counter drugs, whereas active vitamin D₃ preparations (eg, alphacalcidol) are strictly regulated substances. They do not require activation by 1,α-hydroxylase, whether renally or extrarenally expressed, to exert their genomic and nongenomic effects. In randomized placebo-controlled studies by Lind et al, the administration of alphacalcidol (active vitamin D₃) did not influence the serum levels of both 25-OH-D₃ and 1,25-(OH)₂-D₃, and decreased blood pressure in healthy individuals but also induced a 50% increase in urinary calcium excretion, which is associated with hypertension in some population studies.⁶³ Administration of active vitamin D₃ in middle-aged, vitamin D₃-replete individuals with impaired glucose tolerance did not produce significant effects on blood pressure, insulin sensitivity, or glucose tolerance, leading to the conclusion that these patients do not benefit from supplementation.⁶⁴ The same authors also demonstrated that the reduction of blood pressure during long-term treatment with active vitamin D₃ is dependent on plasma renin activity and calcium status in patients with essential

hypertension.⁶⁵ The results from the latter study supported the idea of a relationship between calcium metabolism and the renin-aldosterone system in essential hypertension, and suggested a beneficial effect of vitamin D₃ supplementation on blood pressure in patients with low-renin hypertension.⁶⁵ Previous studies, which involved the administration of active vitamin D₃, did not provide information on the extrarenal activation of vitamin D₃ precursors as proposed in the current article.

A possible approach to assess the level of extrarenal activation of circulating precursors of vitamin D₃ to the active hormonal form would necessitate the measurement of calcitriol acid, the metabolite of vitamin D₃, in urine collections, and the assessment of 24-h urinary excretion in comparison with circulating concentrations of 25-OH-D₃, 1,25-(OH)₂-D₃, markers of inflammation, and white blood cell counts. Low circulating concentrations of active vitamin D₃, combined with increased urinary excretion of calcitriol acid, suggest the extrarenal activation of vitamin D₃. Indeed, except for extreme cases of granulomatous infections, extrarenally activated vitamin D₃ is locally metabolized before spilling over into the circulation in its active form.²⁶ Although the in vitro evidence on the local effects of active vitamin D₃ on the arterial wall is convincing, longitudinal research into high-fidelity arterial phenotypes and the possible associations with vitamin D₃ metabolism in various states of inflammation might shed new light on the cardiovascular impact of supplementation and solar exposure.

We propose to take advantage of the natural genetic randomization during sexual reproduction⁶⁶ by studying the possible associations of blood pressure and arterial phenotypes⁶⁷ with functionally active polymorphisms in the enzymes involved in the generation or catabolism of 25-hydroxyvitamin D₃ or 1 α ,25-dihydroxyvitamin D₃, or in signal transduction via the vitamin D₃ receptor.⁶⁸ Alleles controlling the innate activity of vitamin D₃ are randomly transmitted from parents to offspring, so that confounding factors are evenly distributed in subjects who are or are not exposed to higher vitamin D₃ activity. Moreover, this genetic approach would also overcome the problem of reverse causality, because the genotype is determined before the onset of disease. Remarkably, as shown in Table 1, some of the candidate genes involved in the synthetic or catabolic pathways of vitamin D₃ or in mediating its activity are located in chromosomal regions previously linked to systolic or diastolic blood pressure,^{69–71} mean arterial pressure,⁷² pulse pressure,⁶⁹ or hypertension.^{73,74} Muray et al demonstrated a positive association of blood pressure and circulating levels of 25-OH-vitamin D₃ in men with the BB-*Bsm* I vitamin D-receptor genotype.⁷⁵ Along the lines of this article, several researchers reported associations of myocardial infarction,⁷⁶ coronary heart disease,⁷⁷ and aortic valve stenosis⁷⁸ with genetic variation in the vitamin D₃ receptor.

Table 1. Genes with potential impact on the innate activity of vitamin D, and cardiovascular phenotypes with linkage to similar chromosomal regions

Gene	Genes influencing innate activity of vitamin D			Phenotypes with linkage to similar chromosomal regions		
	Chromosome	Location (cM)*	Function	Phenotype	Marker	Distance (cM)
CYP27A1	2q33-qter	218	D→25-D	SBP ⁶⁹	D2S2297	230.0
CYP3A4	7q21.1	111	D→25-D	SBP ⁶⁹	D7S1799	113.92
				DBP ⁶⁹	D7S1799	111.0
				MAP ⁶⁹	D7S1799	113.92
				PP ⁶⁹	D7S1799	113.92
				HT ⁷³	Not reported	113.92
CYP2R1	11p15.2	22	D→25-D	SBP ⁶⁹	D11S4464	114.0
SC5DL	11q23.3	113	Sterol-5-desaturase	SBP ⁸²	D11S1998	113.13
				DBP ⁸²	D11S1998	113.13
DHCR7	11q13.2-5	73	7-dehydrocholesterol reductase			
Vitamin D receptor	12q13.11	63	Signal transduction			
CYP27B1	12q13.1-q13.3	70	25-D→1 α ,25-D			
SULT2A1	19q13.3	84	Excretion of steroids			
CYP24A1	20q13.2-q13.3	79	A/I vitamin D	SBP ⁸²	Not reported	74.0
LSS	21q22.3	56	Lanosterol synthesis	HT ⁷⁴	Not reported	89.0
				HT ⁷³	Not reported	86.0
				SBP ⁷¹	D21S1446	56.0
						2.02

A/I vitamin D = conversion of 25-hydroxyvitamin D to 24,25-dihydroxyvitamin D and inactivation of 1 α ,25-dihydroxyvitamin D to calcitriol acid; D→25-D = conversion of vitamin D to 25-hydroxyvitamin D; 25-D→1 α ,25-D = conversion of 25-hydroxyvitamin D to 25-dihydroxyvitamin D; DBP = diastolic blood pressure; HT = hypertension; LOD = logarithm of the odds; MAP = mean arterial pressure; PP = pulse pressure; SBP = systolic blood pressure.

* Marshfield map (<http://research.marshfieldclinic.org/genetics>).

Conclusions

Since the introduction of vitamin D₃ supplements in the 1930s to prevent rickets, numerous reports described the symptoms of iatrogenic or accidental overdosage with vitamin D₃.^{26,79,80} The most common symptoms are hypercalcemia; calcinosis around joints; and metastatic calcifications in the arteries, lungs, liver, spleen, and kidneys. Currently, there is no consensus about the recommended daily allowance of vitamin D₃.⁸¹ In several countries, milk and food supplements are fortified with vitamin D₃ on an industrial scale to reduce the risk for hypovitaminosis D₃.²⁶ Maintenance of current sun-avoidance policies to prevent skin cancer while supplementing food with vitamin D₃ might not be the optimal way to balance the risks of inadequate exposure to UVB.⁶¹

Cardiovascular disease is the leading cause of morbidity and mortality in developed and most developing nations. The seemingly beneficial effects on blood pressure seen during short-term supplementation with vitamin D₃ have never been confirmed for its prolonged use. Given the currently reviewed extraskeletal effects of vitamin D₃ and the interaction with inflammatory disease, this "old" topic should be readdressed. Although the beneficial effect of a lifelong calcium-replete status on both bone and cardiovascular health is quite clear, the optimal dose of vitamin D₃ and how to acquire it, are still under discussion. If further evidence confirmed that excessive exposure to oral vitamin D₃ is a risk factor for arterial disease, these findings should instigate a reassessment of the guidelines for the recommended daily allowance, food fortification, use of vitamin D₃ supplementation, and exposure to ultraviolet radiation, in clinical practice.

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